Molecular Therapy
Brief Report

Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer

Solrun Melkorka Maggadottir,[1,2] Svein Dueland,[3] Nadia Mensali,[1] Hanne Hamre,[4] Per Arne Andresen,[5] Marit Renée Myhre,[1] Hedvig V. Juul,[1] Iris Bigalke,[6] Marianne Lundby,[6] Turid Kirsti Hønnåshagen,[6] Stein Sæbøe-Larssen,[6] Dag Josefsen,[6] Trond Hagtvedt,[7] Sébastien Wälchli,[1] Gunnar Kvalheim,[6] and Else Marit Inderberg[1]

[1]Translational Research Unit, Section for Cellular Therapy, Department of Oncology, Oslo University Hospital, Oslo, Norway; [2]Landspitali University Hospital, Reykjavik, Iceland; [3]Department of Oncology, Oslo University Hospital, Oslo, Norway; [4]Department of Oncology, Akershus University Hospital, Lørenskog, Norway; [5]Department of Pathology, Oslo University Hospital, Oslo, Norway; [6]Section for Cellular Therapy, Department of Oncology, Oslo University Hospital, Oslo, Norway; [7]Department of Radiology, Oslo University Hospital, Oslo, Norway

We previously demonstrated the antitumor effectiveness of transiently T cell receptor (TCR)-redirected T cells recognizing a frameshift mutation in transforming growth factor beta receptor 2. We here describe a clinical protocol using mRNA TCR-modified T cells to treat a patient with progressive, treatment-resistant metastatic microsatellite instability-high (MSI-H) colorectal cancer. Following 12 escalating doses of autologous T cells electroporated with *in-vitro*-transcribed Radium-1 TCR mRNA, we assessed T cell cytotoxicity, phenotype, and cytokine production. Tumor markers and growth on computed tomography scans were evaluated and immune cell tumor infiltrate at diagnosis assessed. At diagnosis, tumor-infiltrating CD8+ T cells had minimal expression of exhaustion markers, except for PD-1. Injected Radium-1 T cells were mainly naive and effector memory T cells with low expression of exhaustion markers, except for TIGIT. We confirmed cytotoxicity of transfected Radium-1 T cells against target cells and found key cytokines involved in tumor metastasis, growth, and angiogenesis to fluctuate during treatment. The treatment was well tolerated, and despite his advanced cancer, the patient obtained a stable disease with 6 months survival post-treatment. We conclude that treatment of metastatic MSI-H colorectal cancer with autologous T cells electroporated with Radium-1 TCR mRNA is feasible, safe, and well tolerated and that it warrants further investigation in a phase 1/2 study.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and the second most common cause of cancer-related deaths in 2020 according to the WHO and The Global Cancer Observatory.[1] Despite fair survival for localized CRC, metastatic CRC has a 5-year survival rate of only ∼11%.[2] Mutations in mismatch repair genes lead to microsatellite instability (MSI) and the accumulation of somatic mutations, which can result in the formation of neoantigens. Neoantigens are intriguing targets for adoptive cellular therapy (ACT) since they are exclusively expressed on tumor cells and are not subject to central tolerance in the host immune cells. Thus, neoantigens are considered to make colon cancer with high MSI (MSI-H) more immunogenic than CRC with low MSI (MSI-L). MSI-H is a hallmark of CRC in the autosomal dominant Lynch syndrome (LS), which increases a person's risk of CRC by 40%–80%.[3] In patients without LS, MSI-H is most commonly a sporadic event and is seen in 15% of all CRC cases.[4] MSI-H tumors are enriched in CD8+ and CD4+ T cells, as well as other immune cells, compared to other CRCs,[5–7] and thus the relapse rate is lower for localized MSI-H tumors. Metastatic MSI-H tumors have a poor prognosis,[8,9] and although immunotherapy with immune checkpoint inhibitors (ICIs) can provide good antitumor effects, with fewer side effects than chemotherapy, 30%–50% of patients with MSI-H CRC are unresponsive to ICIs.[10,11] A particular inactivating frameshift mutation in transforming growth factor receptor beta receptor 2 (TGFβRII) is seen in 90% of MSI-H and 15% of MSI-L CRC cases.[12–14] We have previously described a cytotoxic T lymphocyte clone specific for this −1A mutation (nucleotides 709–718 of TGFβRII). This T cell clone has an HLA-A2-restricted T cell receptor (TCR), called Radium-1, that we isolated from a patient with long-term clinical response to peptide vaccination in a phase 1 clinical trial.[13] In our pre-clinical studies, we demonstrated that the Radium-1 TCR can redirect T cells against CRC cell lines expressing the TGFβRII frameshift mutation. Furthermore, T cells transduced with the Radium-1 TCR significantly reduced the growth of CRC and enhanced survival in a xenograft NOD/SCID mouse model.[14]

Received 21 January 2024; accepted 3 April 2024;
https://doi.org/10.1016/j.ymthe.2024.04.009.

Correspondence: Else Marit Inderberg, Translational Research Unit, Section for Cellular Therapy, Department of Oncology, Oslo University Hospital, Oslo, Norway.
E-mail: elsin@rr-research.no

Please cite this article in press as: Maggadottir et al., Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer, Molecular Therapy (2024), https://doi.org/10.1016/j.ymthe.2024.04.009

Here, we report on the first-in-human use of the Radium-1 TCR in a patient with LS and advanced metastatic MSI-H CRC, using mRNA electroporation for transient TCR expression to reduce the risk of off-tumor toxicity.[14,15] The treatment was well tolerated with stabilization of tumor size, and the patient surpassed our conceived life expectancy. To the authors' knowledge, this is the first clinical report of mRNA-based TCR therapy targeting a neoantigen in solid cancer.

In summary, this brief report supports the safety and feasibility of mRNA TCR T cell therapy targeting neoantigens in patients with solid malignancies, as well as confirms the applicability of TCRs identified in patients responding to therapeutic cancer vaccines.

RESULTS

Case presentation

A 42-year-old patient with LS was diagnosed with colorectal MSI-H adenocarcinoma with metastases in the liver. The disease progressed following subtotal colectomy despite chemotherapy but stabilized and then continued with a slow progression on pembrolizumab for 35 cycles over 2 years. He was kept on ICIs given his historical progression on chemotherapy and scarce treatment options. Thereafter, his disease progressed rapidly in concordance with data demonstrating limited ICI response in CRC with liver metastasis.[16] At enrollment, 2 months after ICIs were discontinued, the patient had developed a new lesion in the lung, widespread metastasis in the liver causing intrahepatic cholestasis, and portal vein compression leading to splenic enlargement and compression of the inferior vena cava. In addition, metastases were found endoluminally in the gastrointestinal tract as well as in lymph nodes and peritoneum/retroperitoneum. The patient received 12 systemic infusions of Radium-1-electroporated T cells over 6 weeks (Figures 1A and S1), with prior confirmation of target antigen specific cytotoxicity and tumor necrosis factor α (TNF-α)/interferon γ (IFN-γ) cytokine production (Figures 1B, 1C, and S2). In general, the target cells were loaded with peptide to make sure the response in the TCR-electroporated T cells was detected in a strong and consistent matter, but the T cells also recognized the non-peptide-loaded HLA-A2+ HCT 116 cell line, which endogenously expresses the mutated TGFβRII antigen, both with and without prior exposure to IFN-γ (Figure S2). The treatment was well tolerated, with only minimal side effects of low-grade fever immediately following the infusions, and no significant toxicities were encountered.

Phenotypic description of the Radium-1 TCR T cells

According to flow cytometric analysis, the phenotype of the expanded and electroporated Radium-1 T cells were composed of around twice as many CD8 T cells as CD4 T cells (Figure 1D) and mainly corresponded to naive T (T_N) cells and effector memory T (T_{EM}) cells with fewer central memory T (T_{CM}) cells (Figure 1E). Comparable results were found for control T cells electroporated with water (data not shown). Cellular viability was 82%–95% for all infusion doses. Less than 10% of the infused Radium-1-electroporated T cells expressed PD1, TIM3, or LAG3, whereas a higher proportion, or 28.3% of CD4+ T cells and 37.1% of CD8+ T cells, expressed TIGIT (Figure 1F). Additionally, a high proportion of CD8+ T cells expressed CD39 and CD73 T regulatory/exhaustion markers, as opposed to only a small fraction of CD4+ T cells (Figure 1F).

Cellular analysis of the tumor tissue prior to treatment

T cell profiling of a tumor biopsy from the time of diagnosis, 3 years prior to TGFβRII adoptive cellular therapy (ACT), demonstrated that a third to half of the T cells found in the tumor tissue were CD8+ and were more concentrated in the parenchyma than the stroma. Although the majority of the CD8+ tumor-infiltrating T cells did not express LAG3 or TIM3 exhaustion markers, a small proportion expressed PD1, and this was also more pronounced in the parenchyma (Figures S3 and S4).

Radium-1 T cell infusions were well tolerated with no severe side effects, only low-grade fever and chills on days 1–2 after infusions. No severe side effects were observed, nor were there any reactions necessitating pausing, pre-medication, dose adjustment, or other changes in the planned infusion schedule.

General blood status and metabolic panels were stable throughout the treatment course. Due to large liver metastases with dilation of bile ducts from compression and increasing pruritus, rising bilirubin, and cholestasis, the patient received intrahepatic and external stents halfway through his ACT. He responded promptly with decreased bilirubin and pruritus. The bilirubin slowly continued to decrease during Radium-1 infusions and after completion of treatment.

Plasma cytokine responses to Radium-1 T cell infusions

During the Radium-1 T cell infusions, an interesting peripheral blood profile of pro- and antitumor cytokines was demonstrated. Most notably, CXCL10, or human IFN-inducible protein-10, was significantly upregulated, although it decreased to baseline by the time of the last infusion (see Figure 2A). Additionally, two antitumor cytokines, interleukin (IL)-2 and IL-12, as well as the pleiotropic cytokine IL-10, increased slightly initially and then decreased to prior levels, only to increase again during the last week of treatment and post-treatment completion (see Figure 2B). Pro-tumor growth factors PDGF-BB, CCL5 (RANTES), and VEGF were downregulated during the treatment period and then significantly upregulated after treatment completion (Figures 2C and 2D). IL-8 (CXCL8) levels increased initially, then declined to baseline, but rose again around treatment completion (see Figure 2D).

Antitumor activity of Radium-1 T cell treatment

The size of four different tumor lesions was monitored by computed tomography (CT). A mixed tumor response was observed in the individual lesions, but the patient had stable disease according to RECIST 1.1 criteria when pre-treatment CT was compared to the CT 10 weeks post-treatment completion (see Figure 2E and Table S1). This was

Please cite this article in press as: Maggadottir et al., Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer, Molecular Therapy (2024), https://doi.org/10.1016/j.ymthe.2024.04.009

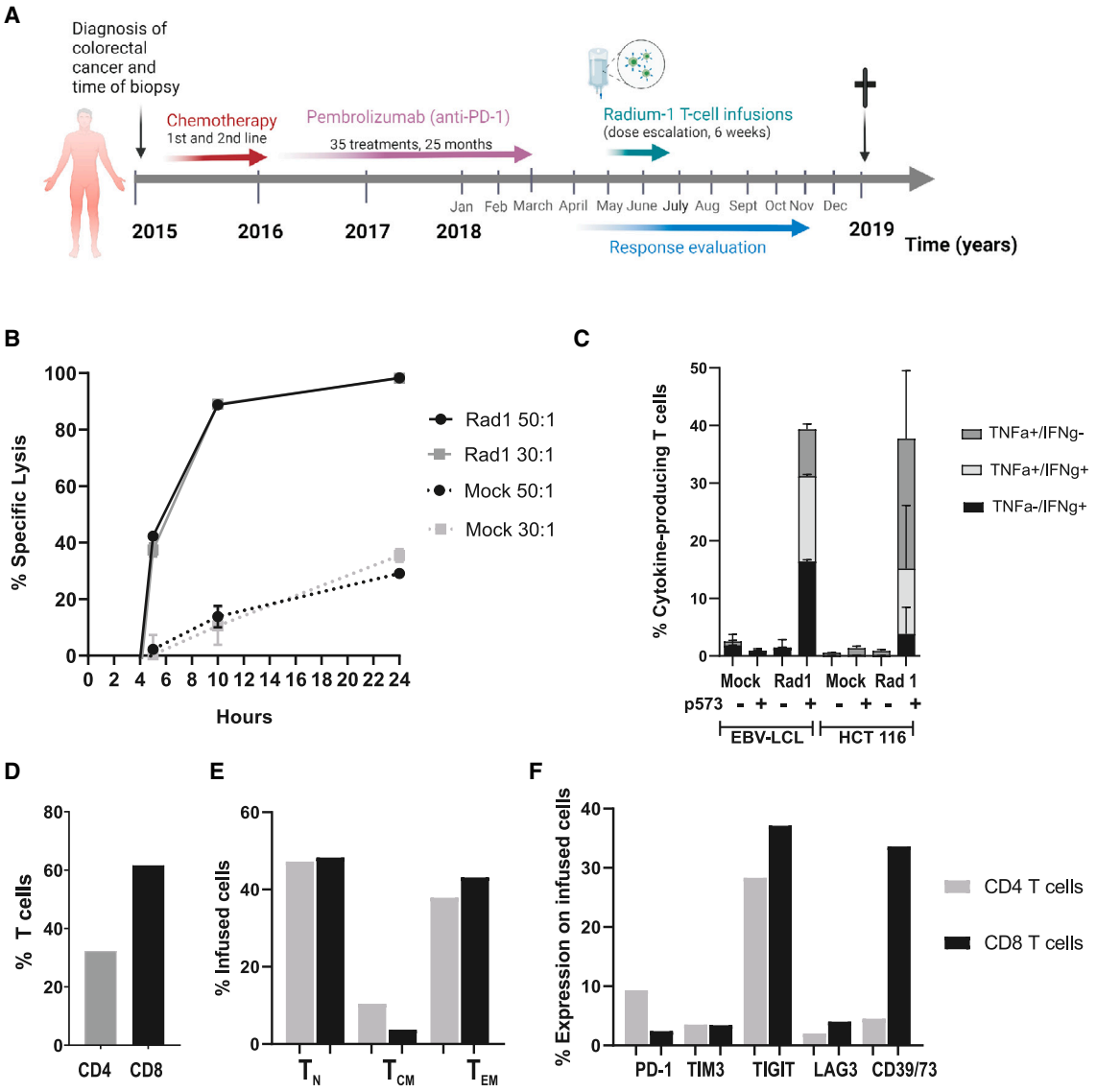

Figure 1. Clinical course of the patient and T cell characterization

(A) The patient had a subtotal colectomy for CRC in late 2015. He first underwent 4 courses of FLIRI-Vectibix (5-fluorouracil, irinotekan, calciumfolinat, panitumumab) and then 4 courses of FLOX-Avastin (5-fluorouracil, leucovorin, oxaliplatin, bevacizumab). The tumor progressed on both treatment regimens. The patient then received 35 doses of pembrolizumab (200 mg every 3 weeks), during which the tumor continued to progress but more slowly. 2 months after stopping pembrolizumab, the patient started infusions of Radium-1 (Rad-1)-transfected autologous T cells. The patient received 12 doses over the course of 6 weeks of increasing cellular doses. The patient succumbed to his disease 6 months after completion of T cell infusions. Created in BioRender.com. (B) Rad-1 TCR-transfected T cells and mock-electroporated T cells were co-incubated with the luciferase expressing colon cancer cell line HCT 116. Target cells were loaded with 5 μM of the 573 peptide (p573) and cytotoxicity measured by bioluminescence. Results are from dose 2, treatment week 5. Effector:target (E:T) ratios were 30:1 and 50:1. Plotted values represent mean ± standard deviations. (C) Rad-1 TCR-transfected T cells were incubated for 5 h with target cell lines HCT 116 or EBV-LCL loaded or not with 5 μM peptide (p573) at an E:T ratio of 1:2. Mock-electroporated T cells were used as controls. Intracellular staining for TNF-α and IFN-γ are shown for CD8+ T cells. Plotted values represent mean ± standard deviations. (D–F) Expanded T cells electroporated with Rad-1 were phenotyped by flow cytometry. (E) Proportions of CD4+ and CD8 T cells, (E) proportions of T cell subsets: naive T (T_N) cells are CCR7+, CD45RA+, and CD62L+; central memory T (T_{CM}) cells are CCR7+, CD45RO+, and CD62L+; and effector memory T (T_{EM}) cells are CCR7−, CD45RO+ and CD62L−. (F) Expression of exhaustion and regulatory markers.

supported by stable CEA (carcinoembryonic antigen) tumor marker values (see Figure 2F). CT at 5 weeks post-treatment completion demonstrated air bubbles in one liver and one peritoneal metastasis. This increased at 10 weeks but decreased again at 19 weeks. Evaluation of Hounsfield units (HUs) in the metastasis suggested necrosis at all time points and a moderate increase over time as shown by

Please cite this article in press as: Maggadottir et al., Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer, Molecular Therapy (2024), https://doi.org/10.1016/j.ymthe.2024.04.009

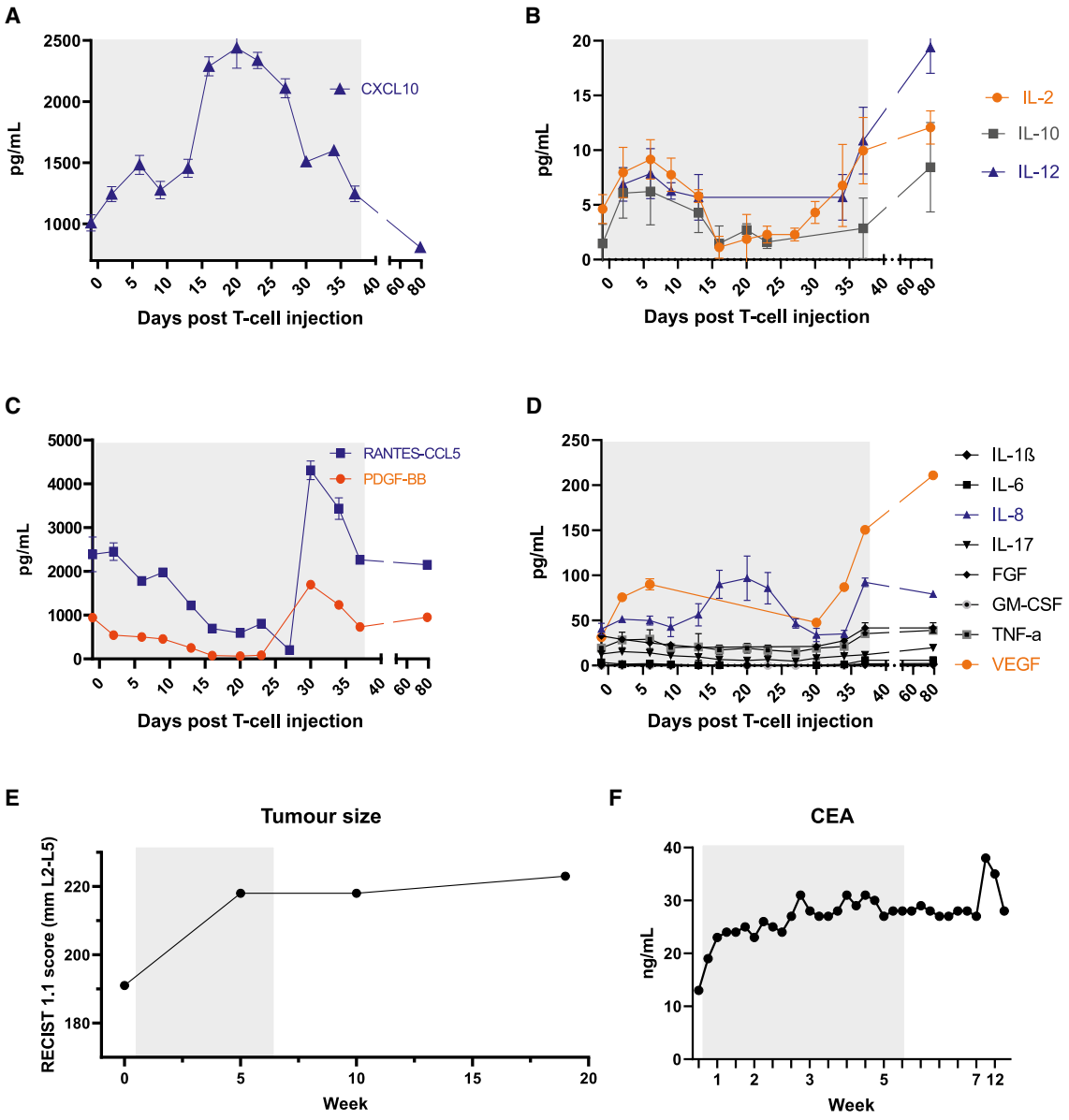

Figure 2. Peripheral blood cytokine levels and tumor response

(A–D) Using Human Cytokine 27-plex Assay, we evaluated the cytokine profile in peripheral blood at baseline, during the Rad-1 TCR infusions, and at 6 weeks after treatment completion. The graphs show the indicated cytokines, and pale gray boxes indicate the T cell treatment period. (E) CT scans were obtained 9 days prior to initiation of Rad-1 infusions as well as at 5, 10, and 19 weeks post-treatment completion. RECIST 1.1 response criteria were used to determine objective tumor response. 4 target lesions were measured at all time points (L2–L5). Details on target lesion size and origin are shown in Table S1. (F) The colon cancer marker CEA (carcinoembryonic antigen) was measured in serum at regular intervals from just prior to treatment until 6 weeks after completion. Pale gray boxes indicate the T cell treatment period. Plotted values represent mean ± standard deviations.

decreasing HUs (Table S2). Bile cultures grew pseudomonas prior to and around the time of imaging at 10 weeks after treatment completion.

The patient survived past our estimated survival based on his extensive, chemo-refractory, and progressive disease. He succumbed to his disease 6 months (190 days) after completion of his Radium-1 TCR infusion treatment.

DISCUSSION

Despite the effectiveness of chimeric antigen receptors (CARs) in hematological malignancies, cellular immunotherapies for solid cancers

Please cite this article in press as: Maggadottir et al., Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer, Molecular Therapy (2024), https://doi.org/10.1016/j.ymthe.2024.04.009

are sorely lacking and none are FDA approved as of yet. TCR therapy has advantages over CARs, namely a broader range of targets, higher antigen sensitivity, reduced risk of off-tumor toxicity and cross-reactivity with normal cells, and the prospect of epitope spreading. Cross-reactivity and affinity-enhanced receptors have proven deadly in previous trials.[17] We propose the use of mRNA electroporation to address this risk by transient TCR expression where serious side effects could be mitigated by stopping T cell infusions, and new data support the feasibility and safety of this approach for a first-in-human T cell therapy.[18]

Here, we confirm viability and *in vitro* efficacy and demonstrate an excellent safety profile of treatment with Radium-1 TCR-transfected T cells in advanced MSI-H CRC. No cross-reactive toxicities were observed, and side effects were minimal. At the time of diagnosis of the patient, a tumor biopsy demonstrated minimal expression of exhaustion markers except for some PD-1 expression. The transfected T cells demonstrated a higher proportion of T_N and T_{EM} versus T_{CM} cells than expected that is likely explained by the patient's advanced disease and therapies. Although a higher proportion of T_{CM} cells is preferred for ACT with transduction, this phenotype is acceptable for mRNA-based therapy. Overall, the expanded T cells expressed low levels of exhaustion markers, although TIGIT expression suggests pre-exhausted T_{EM} cells, likely induced by previous treatments and illness. Furthermore, expression of CD39 and CD73 on transduced CD8 T cells suggests suppressed tumor immune responses.[19] Potent *in vitro* cytotoxicity of the transfected T cells was measured against peptide-loaded target cells. In general, saturating amounts of peptide were loaded to ensure efficient peptide loading, as limited infusion product was available; however, comparable efficacy was demonstrated with 50-fold lower peptide concentrations and against endogenously expressed antigen. Comparing the profile of infused T cells to tumor-infiltrating T cells from the diagnostic biopsy indicates that the infused T cells were affected by prolonged anti-PD-1 treatment, supporting earlier initiation of targeted therapy. Unfortunately, we were unable analyze a follow-up tumor biopsy as planned post-T cell infusion therapy. A biopsy was obtained, but due to a paucity of tumor cells in the biopsy, further analysis was precluded and a second biopsy forfeited due to the patient's wishes. This brief report describes the treatment of a single patient with LS diagnosed with an anti-PD-1-resistant metastatic MSI-H CRC. Such patients are rare, and since the European Medicines Agency recommended extending indications for pembrolizumab to include first-line treatment of metastatic MSI-H CRC in adults, it became difficult to recruit new patients, and the clinical study was therefore closed.

Despite no definite reduction in tumor size, imaging indicated attenuation of progression and stabilization of the disease burden according to RECIST 1.1 for at least 9 weeks post-treatment completion. The patient's survival was impressive, although not unique, given his prior slow progression on second-line chemotherapy. The cytotoxic antitumor effect of Radium-1 treatment could explain the air pockets in metastases and increasing necrosis measured by HUs. Necrosis could also result from tumor bulk, lack of nutrients and/or blood flow, or an infection. A positron emission tomography scan of metabolic activity could have shed light on treatment response. CEA levels remained stable during treatment but increased abruptly following treatment completion. We believe that the temporary rise in bilirubin and pruritus seen was due to tumor compression on intra- and extrahepatic bile ducts. It is unlikely that this represents a direct effect of the Radium-1 infusions.

CXCL10 fluctuations with treatment are consistent with prior data demonstrating its importance for antitumor responses and induction by IFN-γ. CXCL10 has been associated with recurrence, MSI status, and survival in CRC and clinical benefit to mRNA-based TCR therapy in hepatitis B virus (HBV)-related hepatocellular carcinoma.[20] PDGF-BB and VEGF promote angiogenesis and mitosis in CRC.[21] Although VEGF was suppressed during Radium-1 infusions, it was upregulated immediately following treatment completion. The STAT1-CCL5 axis appears to be important for cellular proliferation in CRC,[22] and the decrease of PDGF-BB and CCL5 during Radium-1 infusions coincides with the stabilization of tumor size and CEA levels. IL-8 impacts migration, proliferation, and angiogenesis; its levels in CRC correlate with poor survival,[23] possibly due to resistance to anoikis in cancer cells; and it is involved in a complex feedback loop with VEGF. The reduction in IL-2, IL-10, and IL-12 during Radium-1 treatment that then increased toward the last doses and further post-treatment could be related to epitope spreading with a broadening of the antitumor response causing the increased tumor necrosis seen on CT scans.

In summary, this brief report supports the safety and feasibility of Radium-1 mRNA-electroporated TCR immunotherapy targeting a neoantigen in a solid malignancy. Considering that 15% of CRC is MSI-H and the presumed incidence of the TGFβRII mutation in CRC of differing MSI status, one may assume that about 25% of all patients with CRC could have the mutation. The HLA allele that Radium-1 TCR is restricted to is present in about 50% of White people in Europe and the USA,[24] albeit at a lower rate in those of African and Asian ancestry. Thus, in total, around 10%–13% of patients with CRC could potentially be eligible for the Radium-1 treatment; however, this number could be further reduced based on other patient specifics. Additionally, patients with other MSI-H cancers such as endometrioid carcinoma, ovarian clear cell carcinoma, and gastric cancer could theoretically benefit from this treatment.[25]

Although significant tumor regression was not demonstrated, tumor size stabilization was seen despite advanced disease and with minimal side effects and demonstrates the feasibility of this approach in advanced solid tumor. Due to its excellent safety profile, further study of TCR T cell therapy in patients with less tumor burden is warranted.

MATERIALS AND METHODS

Patient

We enrolled a 42-year-old patient with LS and metastatic CRC into a hospital exemption treatment protocol (ClinicalTrials.gov: NCT03431311) at the Department of Oncology, Cellular Therapy,

Please cite this article in press as: Maggadottir et al., Transient TCR-based T cell therapy in a patient with advanced treatment-resistant MSI-high colorectal cancer, Molecular Therapy (2024), https://doi.org/10.1016/j.ymthe.2024.04.009

Oslo University Hospital (Oslo, Norway). Inclusion criteria required an age >18 years, an Eastern Cooperative Oncology Group (ECOG) performance score of 0–1, adequate end-organ function, and a diagnosis of metastatic colon cancer of MSI-H-positive metastatic colon cancer with a confirmed −1A frameshift mutation within the poly(A) (A10) sequence of the *TGFβRII* gene (nucleotides 709–718). Additionally, the patients had to be of the HLA-A*02:01 genotype with a life expectancy of >3 months. Exclusion criteria included the exclusion of other metastatic malignancies, other antitumor treatment in the last 4 weeks, steroid treatment, pregnancy/lactation, HIV, HBV, HCV, or syphilis infection, or any other significant comorbid disease. The patient signed an informed consent, and the protocol was approved by an ethics committee (REC approval 2017/1273) and the Norwegian Medicines Agency.

HLA tissue typing

Patient lymphocytes were genotyped for HLA class I and II by the National Tissue Typing Laboratory at Oslo University Hospital using next-generation sequencing.

Clinical protocol

The goal of this hospital exemption treatment protocol (ClinicalTrials.gov: NCT03431311) was to demonstrate the safety and feasibility of treating patients with MSI-H-positive CRC by infusion of autologous T cells electroporated with the Radium-1 TCR mRNA specific for the −1A frameshift mutation in the *TGFβRII* gene.

The patient received 12 intravenous infusions evenly distributed over 6 weeks with escalating doses from 1×10^8 to 2×10^9 cells (see Figure S1). Each batch of T cells was thawed and electroporated with mRNA, and fresh, transfected T cells were transported to the ward and infused through a central venous line. Treatment was administered at the Department of Oncology, Radium Hospital, Oslo, Norway.

Cell lines

For the bioluminescence-based cytotoxicity assay to evaluate the quality of the infusion product, we used the colon cancer cell line HCT 116 (CCL-247, ATCC). The HCT 116 cell line was modified to express ffLuc-EGFP as previously described.[13] The cell line was passaged for less than 6 months after purchase. Cell line identity was verified using short tandem repeat profiling by LabCorp. The cell line was free from mycoplasma contamination. For the functional cytokine production assay, we used HCT 116 as well as an HLA-typed Epstein Barr virus-transformed B cell line (EBV-LCL).

Peripheral blood sample collection and processing

Patient blood samples were collected for plasma (cytokine measurements) and isolation of peripheral blood mononuclear cells (PBMCs) according to the clinical protocol.

Samples for plasma were drawn in EDTA tubes and processed within 30 min before storage at −80°C. Blood samples for PBMC isolation were drawn in ACD tubes, and cells were isolated over Ficoll as described previously and stored in liquid nitrogen.[26] Standard CEA measurements were completed at the Radium Hospital clinical laboratory.

Radium-1 TCR T cell production

PBMCs were collected by leukapheresis and processed at the GMP facility of the Department of Oncology, Cellular Therapy at The Norwegian Radium Hospital in Oslo. Lymphocytes were enriched using elutriation (ELUTRA, TerumoBCT). Briefly, lymphocytes were stimulated with CTS Dynabeads CD3/CD28 at the recommended cell: bead ratio and expanded in CellGro GMP DC medium (CellGenix, Freiburg im Breisgau, Germany) supplemented with 5% human AB serum (PAN-Biotech, Aidenbach, Germany), 10 mM mucomyst (Mylan Healthcare, Borgen, Norway), gentamycin 0.05 mg/mL (Sanofi Aventis, Gentilly, France), and 100 U/mL IL-2 (Proleukin, Clinigen Healthcare, Burton-on-Trent, UK) for 10 days using the Xuri Cell expansion system W5 (Cytiva, Marlborough, MA, USA) as previously described.[27] The lymphocytes were frozen in DMSO-containing cryopreservation media in bags in appropriate doses for electroporation and stored in liquid nitrogen. Each batch was thawed, thoroughly washed, and incubated for 2–24 h in culture medium. The cells were then washed again in serum-free medium prior to mRNA electroporation. The expanded T cells were electroporated and transfected *in vitro* with *in-vitro*-transcribed codon-optimized mRNA (clinical grade, produced at Oslo University Hospital, Norway) encoding the Radium-1 TCR. For electroporation, the mRNA or water (mock) was mixed with the T cell suspension at 200 μg/mL and electroporated in a 6 mm gap cuvette using the AgilePulse MAX electroporator system (BTX Technologies, Hawthorne, NY, USA). The electroporation parameters were 770 V for 0.4 ms with 9 pulses in 1,000 ms intervals. Following electroporation, the T cells were left to recover in culture medium containing human serum for 2 h, then washed again to remove the medium and resuspended in the final formulation ready for infusion. Each infused batch was evaluated for standard release criteria including appropriate T cell count and viability and phenotyping by flow cytometry. Each batch was tested for microbiological contamination, and results for electroporated cells were available post-infusion. In addition, TCR expression was confirmed by flow cytometry and cell count performed on each batch prior to release. The cells were only thawed immediately prior to electroporation and infusion.

T cell phenotyping and functional assay

For extracellular staining, T cells were washed in staining buffer (SB) consisting of PBS containing 2% fetal bovine serum (Gibco, Thermo Fisher Scientific, Bristol, RI, USA) before staining for 20 min at room temperature. The cells were then washed in SB and fixed in SB containing 1% paraformaldehyde.

The following antibodies were used: CD4 (BD Biosciences), CD8 (eBiosciences or Life Technology), CCR7 (eBiosciences), CD39 (Invitrogen, Life Technology), CD45RA (eBiosciences), CD45RO (BioLegend), CD62L (BioLegend), CD73 (Invitrogen, Life Technology), PD1 (Invitrogen, Life Technology), TIM3 (BioLegend),

LAG3 (BioLegend), and TIGIT (Invitrogen, Life Technology) for phenotyping assay and CD4, CD8, IFN-γ (Invitrogen, Life Technology), and TNF-α (BD Pharmingen) for cytokine functional assay.

For intracellular staining, Radium-1 TCR-electroporated patient T cells were thawed and rested in medium for 2 h prior to the assay. The T cells were then incubated for 6 h with HCT 116 or EBV-LCL tumor cells loaded with peptide 573 at indicated doses at a 1:2 effector:target (E:T) ratio in the presence of BD GolgiPlug (BD Biosciences, San Jose, CA, USA) and BD Golgistop (BD Biosciences) at a 1/1,000 dilution. Peptide 573 RLSSCVPVA (amino acid sequence 131–139) was synthesized by ProImmune from a TGFβRII frameshift protein resulting from a 1 bp deletion (−1A) in an adenosine stretch (A10) from base number 709–718 of *TGFβRII* (sequence for wild-type human *TGFβRII* is GenBank: NM_003242). Mock-electroporated T cells and PMA/ionomycin stimulation were used as controls. All conditions were run in duplicate. The T cells were stained with anti-CD4, -CD8, -TNF-α, and -IFN-γ antibodies using the PerFix-nc kit according to the manufacturer's instructions (Beckman Coulter, Brea, CA, USA). Plotted values show mean ± standard deviations.

Cells were acquired on a BD FACSCanto flow cytometer (BD Biosciences) and the data analyzed using FlowJo software (Treestar, Ashland, OR, USA).

Bioluminescence-based cytotoxicity assay

Luciferase-expressing HCT 116 tumor cells were loaded overnight with 5 μM of the peptide 573, counted, and resuspended at a concentration of 3×10^5 cells/mL. In experiments with only non-peptide-loaded target cells, HCT 116 were co-cultured with 500 IU/mL of human recombinant IFN-γ (PeproTech, Rocky Hill, NJ, USA) overnight to increase the endogenous peptide presentation.

D-Luciferin (75 μg/mL; PerkinElmer, Waltham, MA, USA) was added to the cells, which were then seeded in 96-well white round-bottomed plates at 100 μL/well in triplicate.

Effector T cells (mock or Radium-1 transfected) were added at E:T ratios of 30:1 or 50:1 unless otherwise stated. To determine spontaneous and maximal killing, wells were seeded with target cells only or with target cells in 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA). The cells were left at 37°C, and the bioluminescence was measured with a luminometer (VICTOR Multilabel Plate Reader, PerkinElmer) as relative light units (RLUs) at indicated time points. Triplicate wells were averaged, and lysis percentage was calculated using the following equation: % specific lysis = 100 × (spontaneous cell death RLU − sample RLU)/(spontaneous death RLU – maximal killing RLU). Plotted values show mean ± standard deviations.

Analysis of plasma cytokines

Plasma was sampled from peripheral blood at indicated time points pre- and post-treatment, and the cytokine levels were measured using the BioplexPro Human Cytokine 27-plex Assay (BioRad Laboratories) according to the manufacturer's instructions. The analysis was performed on the Bio-Plex 200 system instrument from BioRad Laboratories. Plotted values show mean ± standard deviations of duplicates.

CT data analysis

Tumor response was evaluated by CT imaging. The first CT scan was 9 days prior to the first Radium-1 T cell infusion. The second was 5 weeks post-completion of the T cell infusions, and the last two were at 10 and 19 weeks post-treatment completion, respectively. Prior to treatment, CT demonstrated a lesion in the right upper lung and prominent nodules in the fatty tissue surrounding the heart. The patient had widespread liver metastases, with intrahepatic cholestasis caused by tumor mass resulting in significant compression of the portal vein. Spleen was enlarged, likely secondary to portal vein compression. Additionally, there was widespread endoluminal metastasis within the gastrointestinal tract and widespread peritoneal and lymph node metastasis. Since the patient presented with multiple metastatic lesions in different organs prior to therapy, measurements of four target lesions were used to evaluate treatment response. The images were analyzed according to the RECIST 1.1 criteria,[28] with stable disease being a $\leq$20% increase in the RECIST 1.1. sum when comparing the CT scan prior to treatment initiation and the CT scan at 10 weeks post-treatment completion.

HU measurement is a universally used unit in CT scanning to display attenuation coefficients based on assigned densities of air and water. Thus, HUs can be used to estimate the density of the tissue. Lower HU values represent increased necrosis or less density of the lesion.

Veracyte data analysis

To assess the immune cell infiltrate in the tumor at time of diagnosis, we sent a tissue specimen to Veracyte in France (Luminy Biotech Enterprises). Veracyte provides immuno-oncologic diagnostics based on immunohistochemistry and spatial tissue molecular information in cancers such as colon cancer.[29] The tumor biopsy specimen was obtained at the time of initial cancer diagnosis, 3 years prior to the start of Radium-1 TCR immunotherapy. Unfortunately, we were unable to analyze follow-up tumor biopsies post-T cell infusion therapy. Pre- and post-treatment biopsies were obtained but dismissed due to a paucity of tumor cells, and the patient declined further biopsies.

DATA AND CODE AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article and its supplemental information.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ymthe.2024.04.009.

ACKNOWLEDGMENTS

We are grateful to Dr. Tormod Guren for pre-screening of patients (REC approval 2017/101) as well as initial planning of the clinical

protocol. We would like to thank study coordinator Signe Øien Fretland and study nurse Kirsti Stray for excellent assistance with the study. We thank our team of engineers, Lisbeth J. Skoge, Guri Solum, Lene Mowinckel, and Imran Aydemir-Ay, for excellent work on T cell expansions and Dr. Jens Andreas Lindin Jørgensen for GMP-grade mRNA preparation. We thank Hedvig Vidarsdotter Juul for the functional testing of electroporated T cells. We are grateful to pharmacist Cecilie Nguyen for assistance with documentation. We would also like to thank the Department of Immunology and Transfusion Medicine, Oslo University Hospital-Rikshospitalet, Oslo, Norway, for their kind help with HLA typing. This study was funded by the Research Council of Norway (grant nos. 244388, 248902, 337468, and 326811) and the South-Eastern Norway Regional Health Authority (grant no. 2017075). We thank the Flow Cytometry Core Facility at the Oslo University Hospital.

AUTHOR CONTRIBUTIONS

G.K., S.D., S.M.M., I.B., and E.M.I. designed and supervised the clinical study. G.K., S.D., and H.H. enrolled the patient and provided patient care. P.A.A. performed tumor mutational analysis for inclusion. S.W. and S.S.-L. supervised and performed GMP-grade mRNA production. I.B., S.S.-L., T.K.H., M.L., D.J., and G.K. performed and supervised TCR T cell production. G.K., S.D., H.H., T.H., and S.M.M. collected and analyzed clinical data. S.M.M., N.M., M.R.M., and H.V.J. performed the experiments and analyses. S.M.M., G.K., S.W., and E.M.I. wrote the manuscript and generated the figures. All authors edited and approved the manuscript.

DECLARATION OF INTERESTS

G.K., S.W., and E.M.I. are inventors of intellectual property (WO2017194555).

REFERENCES

1. Observatory, G.C. (2020). Colorectal cancer fact sheet. https://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf.
2. Mathonnet, M., Vanderstraete, M., Bounaix Morand du Puch, C., Giraud, S., Lautrette, C., Ouaissi, M., Tabchouri, N., Taïbi, A., Martin, R., Herafa, I., et al. (2021). ONCOGRAM: study protocol for the evaluation of therapeutic response and survival of metastatic colorectal cancer patients treated according to the guidelines of a chemosensitivity assay, the Oncogramme®. Trials *22*, 556. https://doi.org/10.1186/s13063-021-05531-y.
3. International Mismatch Repair Consortium (2021). Variation in the risk of colorectal cancer in families with Lynch syndrome: a retrospective cohort study. Lancet Oncol. *22*, 1014–1022. https://doi.org/10.1016/S1470-2045(21)00189-3.
4. Boland, C.R., and Goel, A. (2010). Microsatellite instability in colorectal cancer. Gastroenterology *138*, 2073–2087.e3. https://doi.org/10.1053/j.gastro.2009.12.064.
5. Nagorsen, D., Voigt, S., Berg, E., Stein, H., Thiel, E., and Loddenkemper, C. (2007). Tumor-infiltrating macrophages and dendritic cells in human colorectal cancer: relation to local regulatory T cells, systemic T-cell response against tumor-associated antigens and survival. J. Transl. Med. *5*, 62. https://doi.org/10.1186/1479-5876-5-62.
6. Alexander, J., Watanabe, T., Wu, T.T., Rashid, A., Li, S., and Hamilton, S.R. (2001). Histopathological identification of colon cancer with microsatellite instability. Am. J. Pathol. *158*, 527–535. https://doi.org/10.1016/S0002-9440(10)63994-6.
7. Gajewski, T.F., Schreiber, H., and Fu, Y.X. (2013). Innate and adaptive immune cells in the tumor microenvironment. Nat. Immunol. *14*, 1014–1022. https://doi.org/10.1038/ni.2703.
8. Colle, R., Cohen, R., Cochereau, D., Duval, A., Lascols, O., Lopez-Trabada, D., Afchain, P., Trouilloud, I., Parc, Y., Lefevre, J.H., et al. (2017). Immunotherapy and patients treated for cancer with microsatellite instability. Bull. Cancer *104*, 42–51. https://doi.org/10.1016/j.bulcan.2016.11.006.
9. Venderbosch, S., Nagtegaal, I.D., Maughan, T.S., Smith, C.G., Cheadle, J.P., Fisher, D., Kaplan, R., Quirke, P., Seymour, M.T., Richman, S.D., et al. (2014). Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. Clin. Cancer Res. *20*, 5322–5330. https://doi.org/10.1158/1078-0432.CCR-14-0332.
10. Overman, M.J., Lonardi, S., Wong, K.Y.M., Lenz, H.J., Gelsomino, F., Aglietta, M., Morse, M.A., Van Cutsem, E., McDermott, R., Hill, A., et al. (2018). Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J. Clin. Oncol. *36*, 773–779. https://doi.org/10.1200/JCO.2017.76.9901.
11. Diaz, L.A., Shiu, K.K., Kim, T.W., Jensen, B.V., Jensen, L.H., Punt, C., Smith, D., Garcia-Carbonero, R., Benavides, M., Gibbs, P., et al. (2022). Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. Lancet Oncol. *23*, 659–670. https://doi.org/10.1016/S1470-2045(22)00197-8.
12. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R.S., Zborowska, E., Kinzler, K.W., and Vogelstein, B. (1995). Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science *268*, 1336–1338. https://doi.org/10.1126/science.7761852.
13. Inderberg, E.M., Wälchli, S., Myhre, M.R., Trachsel, S., Almåsbak, H., Kvalheim, G., and Gaudernack, G. (2017). T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reduces in vivo tumor growth. Oncoimmunology *6*, e1302631. https://doi.org/10.1080/2162402X.2017.1302631.
14. Mensali, N., Myhre, M.R., Dillard, P., Pollmann, S., Gaudernack, G., Kvalheim, G., Wälchli, S., and Inderberg, E.M. (2019). Preclinical assessment of transiently TCR redirected T cells for solid tumour immunotherapy. Cancer Immunol. Immunother. *68*, 1235–1243. https://doi.org/10.1007/s00262-019-02356-2.
15. Harrer, D.C., Simon, B., Fujii, S.I., Shimizu, K., Uslu, U., Schuler, G., Gerer, K.F., Hoyer, S., Dörrie, J., and Schaft, N. (2017). RNA-transfection of γ/δ T cells with a chimeric antigen receptor or an α/β T-cell receptor: a safer alternative to genetically engineered α/β T cells for the immunotherapy of melanoma. BMC Cancer *17*, 551. https://doi.org/10.1186/s12885-017-3539-3.
16. Chen, E.X., Loree, J.M., Titmuss, E., Jonker, D.J., Kennecke, H.F., Berry, S., Couture, F., Ahmad, C.E., Goffin, J.R., Kavan, P., et al. (2023). Liver Metastases and Immune Checkpoint Inhibitor Efficacy in Patients With Refractory Metastatic Colorectal Cancer: A Secondary Analysis of a Randomized Clinical Trial. JAMA Netw. Open *6*, e2346094. https://doi.org/10.1001/jamanetworkopen.2023.46094.
17. Linette, G.P., Stadtmauer, E.A., Maus, M.V., Rapoport, A.P., Levine, B.L., Emery, L., Litzky, L., Bagg, A., Carreno, B.M., Cimino, P.J., et al. (2013). Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood *122*, 863–871. https://doi.org/10.1182/blood-2013-03-490565.
18. Shah, P.D., Huang, A.C., Xu, X., Orlowski, R., Amaravadi, R.K., Schuchter, L.M., Zhang, P., Tchou, J., Matlawski, T., Cervini, A., et al. (2023). Phase I Trial of Autologous RNA-electroporated cMET-directed CAR T Cells Administered Intravenously in Patients with Melanoma and Breast Carcinoma. Cancer Res. Commun. *3*, 821–829. https://doi.org/10.1158/2767-9764.CRC-22-0486.
19. Kim, M., Min, Y.K., Jang, J., Park, H., Lee, S., and Lee, C.H. (2021). Single-cell RNA sequencing reveals distinct cellular factors for response to immunotherapy targeting CD73 and PD-1 in colorectal cancer. J. Immunother. Cancer *9*, e002503. https://doi.org/10.1136/jitc-2021-002503.
20. Tan, A.T., Meng, F., Jin, J., Zhang, J.Y., Wang, S.Y., Shi, L., Shi, M., Li, Y., Xie, Y., Liu, L.M., et al. (2022). Immunological alterations after immunotherapy with short lived HBV-TCR T cells associates with long-term treatment response in HBV-HCC. Hepatol. Commun. *6*, 841–854. https://doi.org/10.1002/hep4.1857.
21. Hansen, T.F., Qvortrup, C., and Pfeiffer, P. (2021). Angiogenesis Inhibitors for Colorectal Cancer. A Review of the Clinical Data. Cancers (Basel) *13*, 1031. https://doi.org/10.3390/cancers13051031.

22. Niu, M., Yi, M., Dong, B., Luo, S., and Wu, K. (2020). Upregulation of STAT1-CCL5 axis is a biomarker of colon cancer and promotes the proliferation of colon cancer cells. Ann. Transl. Med. *8*, 951. https://doi.org/10.21037/atm-20-4428.

23. Bazzichetto, C., Milella, M., Zampiva, I., Simionato, F., Amoreo, C.A., Buglioni, S., Pacelli, C., Le Pera, L., Colombo, T., Bria, E., et al. (2022). Interleukin-8 in Colorectal Cancer: A Systematic Review and Meta-Analysis of Its Potential Role as a Prognostic Biomarker. Biomedicines *10*, 2631. https://doi.org/10.3390/biomedicines10102631.

24. Middleton, D., Menchaca, L., Rood, H., and Komerofsky, R. (2003). New allele frequency database: http://www.allelefrequencies.net. Tissue Antigens *61*, 403–407. https://doi.org/10.1034/j.1399-0039.2003.00062.x.

25. Mestrallet, G., Brown, M., Bozkus, C.C., and Bhardwaj, N. (2023). Immune escape and resistance to immunotherapy in mismatch repair deficient tumors. Front. Immunol. *14*, 1210164. https://doi.org/10.3389/fimmu.2023.1210164.

26. Lilleby, W., Gaudernack, G., Brunsvig, P.F., Vlatkovic, L., Schulz, M., Mills, K., Hole, K.H., and Inderberg, E.M. (2017). Phase I/IIa clinical trial of a novel hTERT peptide vaccine in men with metastatic hormone-naive prostate cancer. Cancer Immunol. Immunother. *66*, 891–901. https://doi.org/10.1007/s00262-017-1994-y.

27. Almåsbak, H., Rian, E., Hoel, H.J., Pulè, M., Wälchli, S., Kvalheim, G., Gaudernack, G., and Rasmussen, A.M. (2011). Transiently redirected T cells for adoptive transfer. Cytotherapy *13*, 629–640. https://doi.org/10.3109/14653249.2010.542461.

28. Eisenhauer, E.A., Therasse, P., Bogaerts, J., Schwartz, L.H., Sargent, D., Ford, R., Dancey, J., Arbuck, S., Gwyther, S., Mooney, M., et al. (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*, 228–247. https://doi.org/10.1016/j.ejca.2008.10.026.

29. Veracyte (2021). What is Immunoscore. https://io.veracyte.com/immunoscore-in-localized-colon-cancer-for-hcp/.